Molecular Characterization of Vancomycin-resistant Enterococci at University Hospitals in Brno (Czech Republic)

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OBJECTIVES

- Monitoring the incidence of VRE at the particular university hospitals in Brno (Czech Republic) in the period January–September 2015 where the increasing trend of VRE has occurred
- Determination of virulence factors, detection of genes for antimicrobial resistance and assessment of their clonality

BACKGROUNDS

Enterococci are Gram-positive bacteria occurring as part of the natural microflora in the gastrointestinal tract. Vancomycin-resistant enterococci (VRE) represent a growing threat in hospital-acquired infections.

Virulence factors genes

Esp (enterococcal surface protein)	Associated with the biofilm formation, increased virulence, colonization and persistence in urinary tract
GelE (gelatinase)	Hydrolysis of collagen, gelatin and small peptides, associated with endocarditis in animal models
Asa1 (aggregation substance)	Improving of adherence of Enterococci to endocardial cells and renal tubular cells
CylA (cytolysin)	Significantly aggravates the symptoms of endocarditis in the animal model
Hyl (hyaluronidase)	Cleaves the hyaluronic acid contained in the intracellular sealant and enables the spread of microbes and their metabolic products into tissues

METHODS

All samples were collected between January 2015 and September 2015 at university hospitals in Brno (St. Anne's University Hospital, Brothers of Charity Hospital and Masaryk Memorial Cancer Institute). Enterococci were identified using standard microbiological methods and MALDI-TOF MS. Determination of antibiotic susceptibility was performed by disk diffusion test. Re-suspended pure cultures were isolated by automatic system croBEE NA16 (GeneProof, Czech Republic). *VanA* and *vanB* genes were determined by GeneProof VRE PCR Kit (GeneProof, Czech Republic). Genes for virulence factors were examined by end-point PCR according to Vankerckhoven et al. (2004). The clonality of individual strains was determined by pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested DNA.

RESULTS

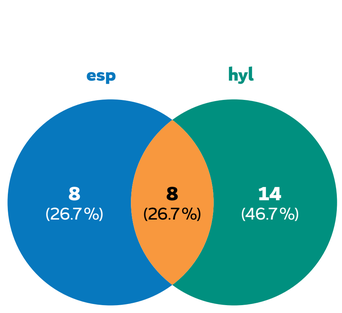

Figure 1. Virulence factors of *E. faecium*. None of virulence factors were detected in two enterococci.

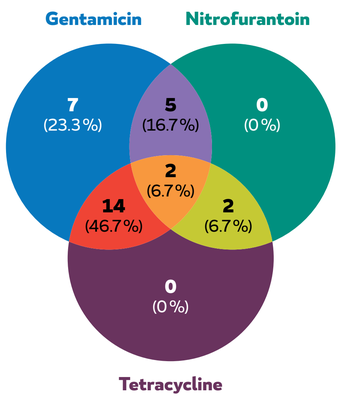

Figure 2. All isolates were resistant to vancomycin and ampicillin and sensitive to tigecycline and linezolid. Venn diagram presents number of strains resistant to gentamicin, nitrofurantoin and tetracycline.

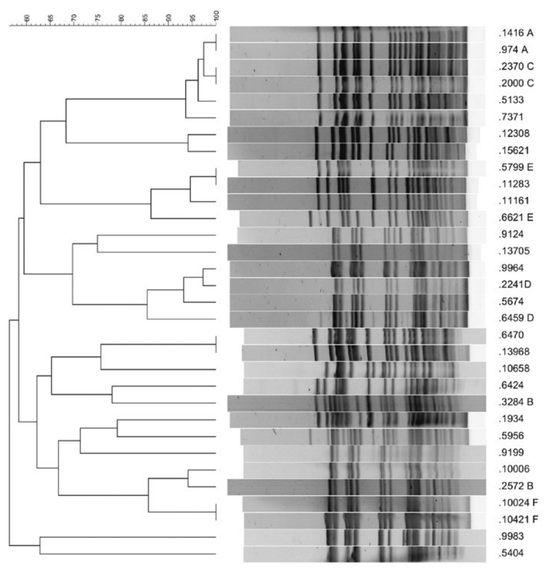

Figure 3. Dendrogram. PFGE did not reveal any significant distribution of identical strains.

Number of isolate	*Hospital/ department**	*Time of isolation*	*Cilincal material*	*Resistance phenotypes*	*Genotype vanA/vanB*	*Virulence gene*
974A	SAUH/INT	01/2015	urine	GEN, FUR	*vanA*	–
1416A	SAUH/INT	01/2015	urine	GEN, FUR	*vanA*	–
3284B	SAUH/INT	02/2015	urine	FUR, TET	*vanA*	*esp, hyl*
1934	SAUH/CKTCH	02/2015	urine	FUR, TET	*vanA*	*esp, hyl*
2000C	SAUH/SUR	02/2015	urine catheter	GEN	*vanA*	*esp*
2572B	SAUH/INT	02/2015	urine	GEN, TET	*vanA*	*hyl*
2370C	SAUH/ARO	02/2015	punctate	GEN	*vanA*	*esp*
5133	SAUH/INT	03/2015	urine	GEN	*vanA*	*esp*
5674	SAUH/INT	03/2015	urine	GEN, FUR, TET	*vanA*	*hyl*
2241D	SAUH/INT	03/2015	blood	GEN, TET	*vanA*	*hyl*
6459D	SAUH/INT	03/2015	perineum swab	GEN, TET	*vanA*	*hyl*
7371	SAUH/CA	04/2015	urine	GEN	*vanA*	*esp,hyl*
5404	SAUH/CKTCH	04/2015	urine	GEN, FUR, TET	*vanA*	*hyl*
5799E	SAUH/CKTCH	04/2015	sternum swab	–	*vanA*	*esp, hyl*
9124	SAUH/INT	04/2015	urine	GEN, TET	*vanA*	*esp*
6424	SAUH/ARO	05/2015	endotracheal aspirate	GEN, TET	*vanA*	*esp, hyl*
6621E	SAUH/CKTCH	05/2015	sternum swab	–	*vanA*	*esp, hyl*
10658	SAUH/INT	05/2015	bilin	GEN, TET	*vanA*	*hyl*
6470	SAUH/SUR	05/2015	lesion swab	GEN	*vanA*	*esp*
5956	MMCI	05/2015	urine	GEN, TET	*vanA*	*hyl*
9199	BCH	06/2015	penis swab	GEN, TET	*vanA*	*hyl*
9983	SAUH/CKTCH	07/2015	central venosis catheter	GEN, TET	*vanA*	*hyl*
9964	SAUH/CKTCH	07/2015	central venosis catheter	GEN, TET	*vanA*	*hyl*
12968	SAUH/INT	07/2015	urine	GEN	*vanA*	*esp*
10006	SAUH/CKTCH	07/2015	central venosis catheter	GEN, TET	*vanA*	*hyl*
10421F	SAUH/ORT	08/2015	urine	GEN, TET	*vanA*	*hyl*
10024F	SAUH/ORT	08/2015	urine	GEN, TET	*vanA*	*hyl*
15621	SAUH/URO	08/2015	urine	GEN, FUR	*vanA*	*esp*
11161	BCH	08/2015	urine	GEN, FUR	*vanA*	*esp, hyl*
11283	BCH	08/2015	urine	GEN, FUR	*vanA*	*esp, hyl*
12308	SAUH/DERM	08/2015	groin swab	GEN	*vanA*	*esp*
13705	BCH	09/2015	stomy	GEN, TET	*vanA*	*hyl*

Table 1. Antimicrobial resistance profiles, distribution of resistance and virulence determinants. **GEN:** gentamicin, **TET:** tetracycline, **FUR:** nitrofurnatonin.

* St. Anne's University Hospital (SAUH), Brothers of Charity Hospital (BCH), Masaryk Memorial Cancer Institute (MMCI), Centre of Cardiovascular and Transplantation Surgery (CKTCH), Department of Internal medicine (INT), Surgical clinic (SUR), Orthopedic surgery (ORT), Department of urology (URO), Dermatovenerology department, First department of cardioangiology (CA)

CONCLUSIONS

- All isolates were identified as *Enterococcus faecium* with *vanA* gene
- The most common clinical material was urine
- Only *esp* and *hyl* were detected
- All isolates were resistant to vancomycin and aminopenicillin and sensitive to linezolid and tigecycline
- PFGE revealed identical strains with possible clonal spreading only in four patients
- PFGE did not reveal any significant distribution of identical strains within university hospitals in Brno. Increased occurrence is probably due to pan-European trends and not bad antibiotic policy at University Hospitals in Brno

Vankerckhoven V., *et* al. (2004), Development of a Multiplex PCR for the Detection of *asa1*, *gelE*, *cylA*, *esp* and *hyl* Genes in Enterococci and Survey for Virulence Determinants among European Hospital Isolates of *Enterococcus faecium*. J. Clin. Micro. 42,4473-4479

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